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# HOW TO

# Collect Field Samples and Identify the Oak Wilt Fungus in the Laboratory

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*Prepared by* Forest Service

**Northeastern Area** State & Private Forestry

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Collecting symptomatic red oak branch samples for oak wilt testing.



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# HOW TO

## Collect Field Samples and Identify the Oak Wilt Fungus in the Laboratory

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## Introduction

Oak wilt is a serious disease that infects many species of oak. It is responsible for the death of thousands of oak trees in forests, woodlots, and home landscapes each year. Oak wilt occurs in the eastern U.S., in an area delimited by Minnesota, Texas, Pennsylvania, and South Carolina.

Oak wilt is caused by a fungus that invades and impairs the tree's water conducting system, resulting in branch wilting and tree death. Oak species vary in their susceptibility to oak wilt. Red oaks are very susceptible; live oaks in Texas are intermediate; and white and bur oaks are moderately resistant to this disease.

Oak trees can become infected by the oak wilt fungus by root systems that become grafted between infected and healthy trees, or by beetle vectors carrying spores to freshly wounded trees. The biology and control of oak wilt is discussed in a USDA Forest Service companion publication, "How To Identify, Prevent, and Control Oak Wilt."

Accurate diagnosis of oak wilt is essential to proper management and is often needed to justify the cost of control efforts. Trained professionals can often diagnose oak wilt in the field based on signs, symptoms, pattern of spread, rate of tree mortality, and site history. Laboratory testing to positively confirm the presence of oak wilt is necessary, however, if field data are inconclusive.

Positive confirmation of oak wilt is obtained by laboratory isolation of the fungus from infected tree tissue. The following information, compiled from published papers and personal communications with diagnosticians in university plant disease clinics, provides standard and currently recommended sample collection and laboratory isolation techniques. Written for field and laboratory staff, it is offered as a practical guide on how to collect branch and stem samples suitable for oak wilt testing, and how to isolate and identify the oak wilt fungus in culture.

## Sample Collection and Storage

Sample quality is paramount to the success of laboratory testing, and results are only as reliable as the sample itself. Improperly collected, stored, or shipped samples may result in a false negative. False negative means the tree has oak wilt, but the presence of the fungus was not confirmed by laboratory testing. Following the procedures outlined in this section will help to ensure sample quality and increase the likelihood of an accurate diagnosis.

1. Select branches that are partially wilted, with symptomatic leaves progressing from the tip of branches inward to the trunk. Be sure that branches are **not** totally wilted, dry, or dead.

2. Collect symptomatic leaves from selected branches. Be sure to package leaf samples separately from branch samples.

   In white and red oaks, look for leaves that are partially wilted and have brown discoloration that moves inward from the leaf margins and progresses from tip to the base of the leaf (Figure 1a,b). In live oak, leaves develop yellow veins that eventually turn brown, producing a symptom called veinal necrosis (Figure 1c).

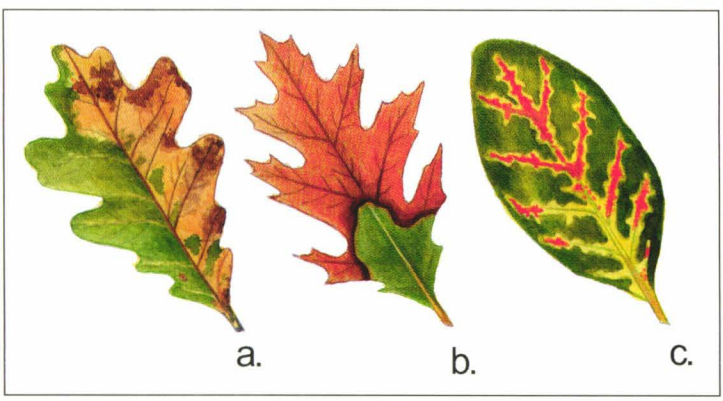


**Figure 1.** *Leaf symptoms of oak wilt.*
*a) White oak showing marginal browning.*
*b) Red oak showing marginal browning.*
*c) Live oak showing veinal necrosis.*

3. Look for discoloration in the sapwood of partially wilted branches. Discoloration may be evident in a cross sectional view of infected branches (Figure 2).

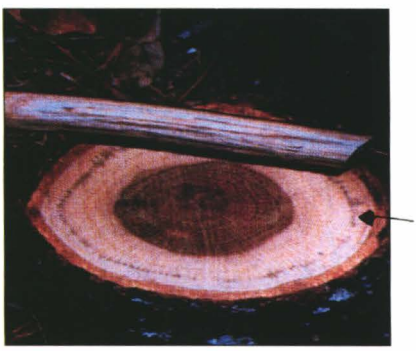

**Figure 2.** *Cross sectional view: black dots of discoloration are evident in the sapwood.*

In some cases, particularly with red oak, discoloration is not evident in a cross sectional view of the branch and is detectable **only** after the bark is removed and the sapwood is longitudinally sectioned (Figure 3).

**Figure 3.** *Longitudinal view: streak of discoloration evident only after removal of the bark.*

In the field, remove the bark from symptomatic branches and check carefully for streaks or flecks of discoloration in the underlying sapwood. If sapwood discoloration is not present in one branch, check other partially wilted branches. Be careful not to remove all of the bark on the branch samples you submit for testing.

4. Select samples from up to three symptomatic branches per single tree. It is critical that the sapwood is moist to the touch, and the inner bark is still alive and green. In the field, check for this by removing the outer bark on a small section of the branch sample (Figure 4). Avoid collecting samples from the extreme tips of branches: this tissue wilts first and is often too dry for successful isolation.

   Branch samples should be at least 1inch in diameter, cut into 6- to 8-inch lengths, and placed in large resealable plastic bags (Figure 5). Smaller diameter branch samples are acceptable **only** if the sapwood is very moist, and samples have been kept cool. Samples from each suspect tree should be labeled separately and placed in individual resealable plastic bags.

5. Bole samples may need to be taken in some cases when symptomatic branches are too high to reach with available equipment. Bole sampling wounds the main trunk of the tree and should be used only when tree health has already been compromised by excessive wilting or heavy defoliation. Bole samples are not the preferred sample type for laboratory testing because the wood is harder and more difficult to chisel out subsamples from, and contamination by saprophytic fungi is more common.

   If bole samples are taken, a "window" must be cut through the outer and inner bark, exposing a clean sapwood surface (Figure 6). Strips of discolored sapwood tissue can then be cut from the outer growth rings with an axe or chisel. If discoloration is not present in the exposed sapwood, check other location(s) on the bole. Avoid including bark in the sample bag because the oak wilt fungus competes poorly with fungal contaminants found on the outer and inner bark.

**Figure 4.** *Remove the bark on a small section of the branch: check that the inner bark is alive and green, and the sapwood is moist to the touch.*

**Figure 5.** *Cut branch samples into 6- to 8-inch lengths and store in a resealable plastic bag.*

**Figure 6.** *A "window" cut through the outer and inner bark, exposing the sapwood.*

6. Keep samples cool during sampling, shipping, and storage, but do not freeze. Sample temperatures should never exceed 85-90° F. Along with resealable plastic bags, be sure to take a cooler and ice packs (avoid ice cubes and dry ice) with you to the field (Figure 7). Never leave samples unrefrigerated or exposed to direct sunlight.

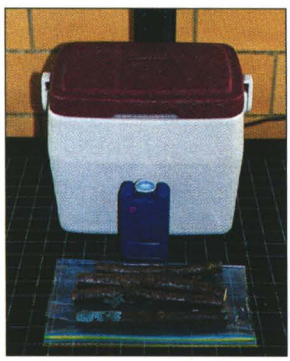

**Figure 7.** *Mandatory field supplies for storing samples.*

7. Provide background information about the tree(s) such as symptoms observed and when they first occurred; age of the tree; proximity to other trees and buildings; any damage caused by storms or other events. Use data collection forms supplied by your local diagnostic laboratory whenever possible.

8. Ship samples by overnight mail or deliver in person to the laboratory. Samples should remain in resealable plastic bags and be shipped in a disposable ice chest with ice packs. Mail samples early in the week to ensure arrival at the laboratory in ample time to culture the sample before the weekend, and to avoid sample delivery on the weekend.

9. Stop collecting samples after annual leaf fall begins in the fall. In Minnesota, the annual cut-off date for oak wilt testing is typically mid- September. In the spring, sampling can begin after the leaves have flushed. If sampling is done during peak infection periods (late April, May, or June in Minnesota), wound dressing should be applied to all pruning cuts.

## Laboratory Isolation Procedures

The following procedures are used routinely in university diagnostic clinics throughout the Midwest and Texas. They are considered to be the best techniques for isolating the oak wilt fungus from branch and stem samples.

1. When samples arrive at the laboratory, keep them in resealable plastic bags and refrigerate immediately. Retain samples in refrigerated storage until culture results are completed, in case there is a need to re-culture the sample because of contamination or other reasons.

2. Surface sterilize branch and stem samples by spraying them with 95% alcohol and then flaming (Figure 8).

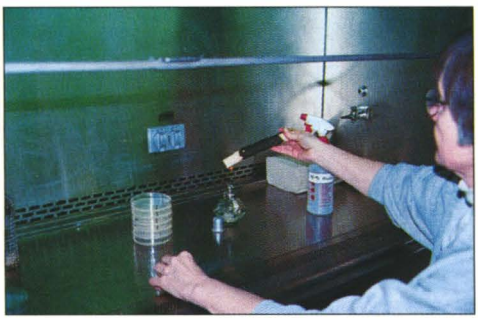

**Figure 8.** *Spraying with 95% alcohol and flaming sterilizes the surface.*

3. Remove the bark on branch samples with a knife that has been sterilized by dipping it in alcohol and flaming.

4. Look carefully for discoloration in the sapwood that appears as streaks or flecks. (Figure 9). If sapwood discoloration is not present in one branch sample, check other branch samples. Be diligent in your search because the likelihood of isolating the oak wilt fungus from discolored sapwood tissue is excellent, nearly 90%. If discoloration is not present, however, the likelihood of isolating the fungus in culture is very low.

**Figure 9.** *Bark peeled back to reveal discoloration in the sapwood.*

5. With a sterile wood gouge or knife, remove strips of discolored sapwood and cut into 1/4- inch pieces.

   **Note:** If the bark and/or strips of sapwood are removed from branch samples outside the sterile environment of the isolation hood, surface disinfect the sapwood strips before cutting them into 1/4-inch pieces and placing them on growth media (Step 6). To disinfect sapwood strips, submerge them in a 10% household bleach solution (1:9 ratio of 5% household bleach to sterile, distilled water) for 1 minute, rinse in sterile distilled water, and blot dry on sterile paper towels.

6. With a sterile forceps, place four sapwood pieces in each of 6 petri plates containing acidified potato dextrose agar (APDA) (Figure 10). See recipe for APDA on page 10.

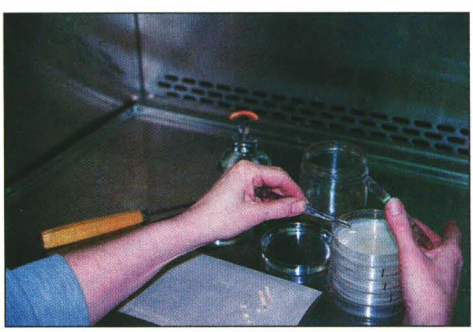

**Figure 10.** *Small pieces of sapwood, cut and transferred with a sterile utensil, are placed on APDA media in a petri dish.*

7. Incubate petri plates at ambient room temperature and lighting. Petri plates are best stored in an incubation hood, but can be stored on the lab bench if sealed with parafilm. Be sure to store plates in a "clean" lab, away from plant samples and other contaminants.

8. The conidial state of the oak wilt fungus, *Chalara,* should be evident in 5-14 days. Mycelial growth of the oak wilt fungus on APDA media first appears nearly transparent, but soon becomes gray, and often has a brown to green cast (Figure 11). Cultures emit a characteristic fruity odor that is easily detected when the petri dish lid is gently opened.

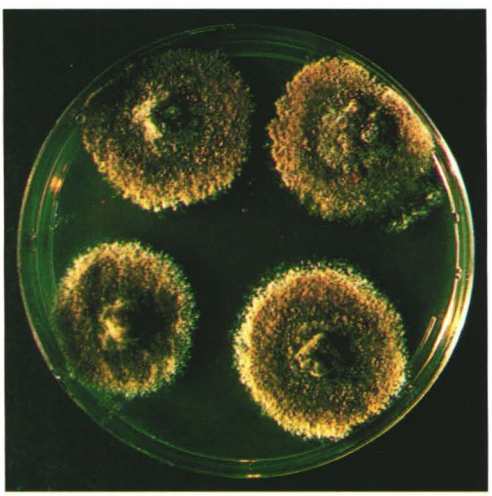

**Figure 11**. *The oak wilt fungus growing on APDA media. Note the brownish-gray color of the colonies.*

A good identifying characteristic of *Chalara* is the presence of endoconidia that are produced within elongated hyphal structures called phialides (Figure 12). Endoconidia are rectangular, greenish when mature, and may be borne in chains.

9. Hold plates for 14 days before interpreting results as negative. Plates should be monitored and read every 2-3 days, and any necessary transfers made.

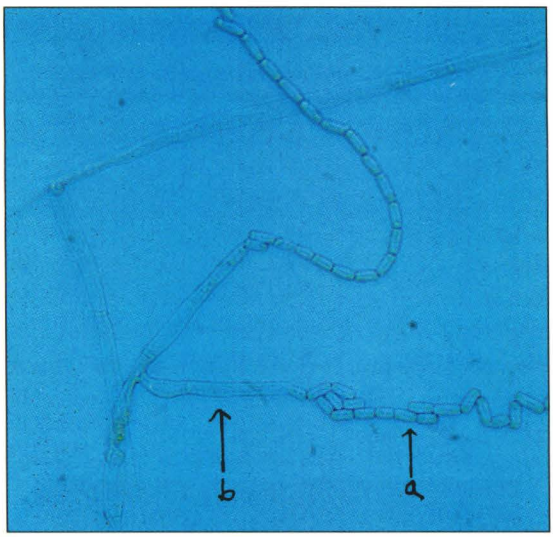


**Figure 12.** *Rectangular endoconidia (a) and elongated phialides (b).*

## Recipe for APDA:

1. Add 39 grams of Difco[R] Commercial PDA (potato dextrose agar) to 1 liter of distilled water.

2. Autoclave for 20 minutes.

3. Add 25% lactic acid at a rate of 5 drops per 100 ml of agar. Add the lactic acid after the agar has cooled, just before you pour the mixture into plates.

4. Store plates inverted, in the refrigerator, to keep water droplets from dripping down on the agar surface.

Mention of trade names does not constitute endorsement by the U.S. Department of Agriculture.

## Companion Publication

O'Brien, J.G.; Mielke, M.E.; Starkey, D.; Juzwik, J. In Press. **How to Identify, Prevent, and Control Oak Wilt.** NA-FR-02-99. USDA Forest Service, Northeastern Area State and Private Forestry. This publication will be available from: USDA Forest Service, St. Paul, MN, 55108 and their web site at http://willow.ncfes.umn.edu

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**For further information, contact:**